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Update on Guard Cells

The Guard Cell-Environment Connection[1]

Ellen V. Kearns and Sarah M. Assmann*

Department of Organismic and Evolutionary Biology, Harvard University, The Biological Laboratories, 16 Divinity Avenue, Cambridge, Massachusetts 02138

This review surveys the signals, intermediate events, and effectors that connect GC swelling and shrinking to environmental conditions. GCs swell when protons are released, hyperpolarizing the plasma membrane (shifting the membrane potential to more negative voltages). This hyperpolarization drives K^+ entry down an electrical gradient through inward K^+ channels. Cl^- influx also occurs, presumably via Cl^-/H^+ symport or Cl^-/OH^- antiport. The entering ions are stored primarily in the vacuole along with malate, which is synthesized during stomatal opening. As intracellular osmotica increase, GCs take up water, balancing their water potential with that of their surroundings, and they begin to swell. Differential cell wall reinforcement bends the swelling GCs outward and widens the pore between them (Willmer, 1983; Taiz and Zeiger, 1991).

Stomatal closure is not the reversal of opening. Anion release and/or Ca^{2+} uptake depolarizes the plasma membrane (shifts the membrane potential to more positive values). This depolarization provides the driving force for K^+ efflux through outward K^+ channels. As levels of K^+ and malate drop, GCs release water and shrink, relaxing the outward bend and closing over the substomatal cavity.

SIGNALS

Signals directing stomatal movement include CO_2, humidity, phytohormones, and blue and red light. Red light is absorbed by Chl, but other receptors have not been definitively identified. The complexity of multiple signals, at times contradictory, must be integrated into a single coherent GC response.

CO_2

Despite the increasing "global change" relevance of plant responses to CO_2, the mechanism of GC CO_2 perception remains a mystery. We do know that stomata respond to CO_2 in both light and darkness. Thus, the response does not require photosynthetic reactions. Stomata respond to C_i, not ambient or stomatal pore CO_2 concentrations (Mott, 1988). Stomatal opening in response to decreasing CO_2 and closure in response to increasing CO_2 help maintain C_i at about 230 μmol mol^{-1} and 100 μmol mol^{-1} in C_3 and C_4 species, respectively (Morison, 1987). In theory, high CO_2 should keep the GCs turgid, since CO_2 is a substrate for both malate synthesis and photosynthetic sugar production. However, high CO_2 apparently contributes to two opposing effects in which turgor loss somehow gains dominance, and the stomata close (Mansfield et al., 1990).

Humidity

Stomata close in response to decreased ambient humidity. Is RH, leaf-air *VPD*, or *E* the signal perceived by the GCs? Stomatal responses in air and helox (79:21 [v/v] helium:oxygen with appropriate additions of water vapor and CO_2) have been compared (Mott and Parkhurst, 1991). In helox, the diffusivity of water vapor is 2.33 times that in normal air. Thus, by switching between air and helox, *E* can be varied independently of *VPD* and vice versa. Stomatal aperture correlated with *E*, rather than with RH or *VPD*. Mott and Parkhurst (1991) suggest that stomatal closure in response to increased *E* simply involves decreased water availability to the GCs and thus decreased GC turgor. However, a GC metabolic response to *E*, or to a signal metabolite carried in the transpiration stream, is also possible.

Phytohormones

More is known about the effects on stomata of ABA and auxins than about the effects of other phytohormones. ABA induces stomatal closure during water stress. In some species the closing is patchy, i.e. the stomata close on some parts of the leaf, but patches of stomata remain open. Patchiness in whole leaves may be caused by a heterobaric vascular arrangement in which sections of the leaf operate autonomously (Mansfield et al., 1990).

ABA stimulates rapid K^+ ($^{86}Rb^+$) efflux from GCs. Additional slow efflux of $^{86}Rb^+$, presumably from the vacuole, requires a time threshold of ABA exposure of 1 to 2 min and is accelerated by increasing external Ca^{2+} from 0.5 to 100 μM (MacRobbie, 1990). ABA-induced stomatal closure sometimes involves Ca^{2+} uptake (Schroeder and Hagiwara, 1990) through nonselective Ca^{2+} channels. These channels have reversal potentials between the equilibrium potentials of Ca^{2+}

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* Corresponding author; fax 1–617–496–5854.

Abbreviations: Ca^{2+}_i, cytosolic Ca^{2+}; C_i, intercellular CO_2 concentration; *E*, transpiration; GC, guard cell; GCP, guard cell protoplast; GDPβS, guanosine 5′-[β-thio]diphosphate; GTPγS, guanosine 5′-[γ-thio]triphosphate; IP_3, inositol-1,4,5-trisphosphate; 1-NAA, 1-naphtylacetic acid; PCRP, photosynthetic carbon reduction pathway; *VPD*, leaf-air vapor pressure difference.

and K^+ and increase Ca^{2+}_i, from resting levels of 0.19 μM to peaks of 0.5 to 5 μM. Unlike animal receptor-ion channel proteins, these channels desensitize and recover without removal of ABA from the external medium. During continuous bathing in 1 μM ABA, Ca^{2+} influx through these channels occurs in repetitive pulses with 10-fold variation in peak Ca^{2+} concentration. This pulsing suggests complex modulation with intermediate steps cycling between channel opening events. Asynchronic channel response or variations in channel conductance may contribute to the phenomenon as well.

Depending on the plant species and the auxin type and concentration, auxins may either promote or inhibit stomatal opening (Davies and Mansfield, 1987). In 5-h incubations of *Vicia faba* epidermal peels, the dose-response curve for stomatal aperture in the presence of 1-NAA is bell shaped, with peak opening at 5 to 10 μM and inhibition of opening at 500 μM (Marten et al., 1991). The inhibition phase of bell-shaped auxin-response curves may be caused by ethylene biosynthesis after 1 to 3 h at high auxin doses (Goodwin and Mercer, 1988). There are conflicting data concerning the effect of ethylene on *V. faba* stomata (Tissera and Ayres, 1986; Levitt et al., 1987).

Auxins modulate anion channel activity when applied in the external bath, but not when applied to the cytosol via a micropipette (Marten et al., 1991). They are also effective on isolated membrane patches. Thus, auxins act on the anion channels via a membrane-delimited pathway. The peak amplitude of current through "R-type" anion channels is shifted by 1-NAA toward more negative voltages (Marten et al., 1991), favoring channel activation at membrane potentials more likely to occur in vivo. Channel opening is likely to be transient because these channels inactivate following stimulation (Marten et al., 1991; Schroeder and Keller, 1992). This transient loss of anions might promote stomatal opening, if channel opening is somehow more important as a "signal" rather than as an effector of decreased GC solute concentration.

Auxins activate plasma membrane H^+-ATPase activity. In patch-clamped GCPs, 5 μM 2,4-D increases an outward current by a few pA. This current presumably results from H^+-ATPase activity, since it is dissipated by dicyclocarbodiimide and requires cytosolic ATP (Lohse and Hedrich, 1992). Pump activation would cause membrane hyperpolarization and drive stomatal opening. Two possibilities arise depending on the type and concentration of auxin. Either pump activation outweighs anion-channel activation, thus resulting in stomatal opening, or anion-channel opening predominates, consistent with stomatal closure. The dual effects of auxins on GCP ion transport may help explain the diversity of auxin effects on stomatal apertures.

Light

Although Chl absorbs both blue and red light, the greater quantum efficiency of blue light in stimulating stomatal opening, particularly at low fluence rates, is the most definitive of several lines of evidence for a GC blue-light receptor (Sharkey and Ogawa, 1987). The identity of the blue-light receptor is unknown.

Two mechanisms have been suggested for blue light-induced H^+ efflux from GCs. One involves a plasma membrane redox chain (Raghavendra, 1990; Gautier et al., 1992) and the other involves an H^+-ATPase (Assmann et al., 1985; Amodeo et al., 1992). After preillumination with 2000 μE m^{-2} s^{-1} red light, *Commelina communis* GCPs release H^+ and take up O_2 in response to a 30-s pulse of 100 μE m^{-2} s^{-1} blue light (Gautier et al., 1992). The required red preillumination can be replaced by 1 mM NADH, suggesting that red light provides a source of electrons for a blue light-driven transport chain. However, other inhibition protocols in this study require the invocation of pools of reduced intermediates awaiting a blue pulse to continue electron flow down the redox chain. Because, in red preilluminated GCPs, electrons flow unimpeded to an artificial electron acceptor, ferricyanide, blue light would have to activate an otherwise dormant electron acceptor, perhaps by a mechanism analogous to the reversible isomerizations caused in phytochromes by red/far red light treatments (Goodwin and Mercer, 1988).

Because of possible solubility and cell entry problems (Schwartz et al., 1991), the lack of vanadate sensitivity in some early studies does not rule out blue light-stimulated H^+-ATPase activity. New protocols show that 500 μM sodium vanadate inhibits blue light-induced swelling in *V. faba* GCPs. Thus, H^+-ATPase involvement is suggested (Amodeo et al., 1992). The amount of charge movement resulting from an ATP-dependent, blue light-stimulated outward current correlates well with that resulting from blue light-stimulated H^+ extrusion (Assmann et al., 1985; Shimazaki et al., 1986; Assmann and Zeiger, 1987). It is interesting that H^+ outward current is stimulated by blue light even in the presence of surplus cytosolic ATP (Assmann et al., 1985; Schroeder, 1988), indicating that blue light initiates a signal transduction chain, rather than just providing ATP via stimulation of photo- and/or oxidative phosphorylation. This signal transduction chain could involve redox processes as regulators of the H^+-ATPase.

Red light, transduced by GC Chl (Sharkey and Ogawa, 1987), is an opening signal for stomata. Red light activation of an outward, hyperpolarizing current is inhibited by the protonophore carbonylcyanide *m*-chlorophenylhydrazone or by cytosolic vanadate (Serrano et al., 1988). The current, which requires cytosolic ATP and Pi, is also abolished by an inhibitor of noncyclic electron transport, DCMU, even in the presence of abundant cytosolic ATP. These results provide evidence that red light-driven chloroplastic reactions activate an electrogenic proton pump at the plasma membrane, and that this activation does not merely reflect increased ATP availability from photophosphorylation (Serrano et al., 1988).

INTERMEDIATE EVENTS

After signal reception, intermediate events lead to an effect. Probable intermediate components of stomatal signal transduction include carbon metabolism, G proteins, Ca^{2+}, IP_3, and phosphatases.

Carbon Metabolism

It has been known for some time that malate production contributes significantly to GC osmotica, especially in illu-

minated GCs (Ogawa et al., 1978). However, the quantitative contribution of photosynthetic carbon metabolism to osmotic buildup is still being debated. Only recently has it been generally accepted that GCs do contain Rubisco. An estimated 2 to 45% of the solute requirements for stomatal opening may be provided by the PCRP (Reckmann et al., 1990; Poffenroth et al., 1992). Poffenroth et al. (1992) present data indicating that light quality (red versus blue) regulates the relative amount of new sugar production from the PCRP versus sugar production from starch breakdown. The amount of sugars may be small but crucial as a "compatible solute" lowering osmotic potential in the relatively small volume of GC cytoplasm.

G Proteins

G proteins are heterotrimeric GTP-binding proteins that transduce excitation of membrane-associated receptors into cellular events. The GTP analog GDPβS locks G proteins in an inactive state, whereas the GTP analog GTPγS locks G proteins in an active state. In *V. faba* GCPs, inward K^+ currents through plasma membrane ion channels are activated by GDPβS and inhibited by GTPγS, implying that activation of a G protein inhibits stomatal opening (Fairley-Grenot and Assmann, 1991). GTPγS does not inhibit K^+ influx in the presence of high concentrations of the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (BAPTA). Thus, the inhibitory effect of an activated G protein on K^+ influx may result in vivo from an associated increase in Ca^{2+}_i, or the inhibitory effect may simply require a physiological Ca^{2+}_i, concentration as a cofactor.

Ca^{2+} and IP_3

Exogenously supplied Ca^{2+} inhibits stomatal opening (Mansfield et al., 1991) by inactivating ion channels mediating K^+ influx (Schroeder and Hagiwara, 1989), possibly via a Ca^{2+}-activated phosphatase (Luan et al., 1993). Ca^{2+} also promotes stomatal closure, activating depolarizing anion channels (Schroeder and Hagiwara, 1989). Effects of Ca^{2+} are complicated by questions concerning its origin. *C. communis* stomata microinjected with caged Ca^{2+} close upon photolytic release of Ca^{2+} into the cytosol (Gilroy et al., 1990). Photolysis of caged IP_3 also stimulates closure and increases Ca^{2+}_i even in the presence of La^{3+}, a Ca^{2+} channel blocker (Gilroy et al., 1990). Thus, IP_3 appears to release Ca^{2+} from internal stores, probably the vacuole or ER (Gilroy et al., 1991). However, Ca^{2+} can also enter the cytosol through three types of plasma membrane channels. GCs have stretch-activated channels with high selectivity for Ca^{2+} over K^+ (Cosgrove and Hedrich, 1991). As discussed previously, channels with significant permeability to both Ca^{2+} and K^+ are also present and can be activated by ABA (Schroeder and Hagiwara, 1990; Cosgrove and Hedrich, 1991). Finally, the inward K^+ channels mentioned above also have limited Ca^{2+} permeability (Fairley-Grenot and Assmann, 1992). It is possible that the relative Ca^{2+} permeability of these channels might be regulated, as is the case for some animal cation channels, and increased by signals that initiate stomatal closure. Whatever the source of Ca^{2+}_i, its accumulation activates channels that mediate anion efflux across the plasma membrane and stimulates the release of other vacuolar ions, such as K^+, which would pass through outward K^+ channels (MacRobbie, 1990).

In *V. faba* GCs, ABA and IP_3 inhibit inward K^+ channels and activate an inward "leak" current that may provide the depolarization required for stomatal closure (Blatt, 1990; Blatt et al., 1990). The carrier of the leak current is unidentified. The leak could result from anion efflux, although its voltage regulation distinguishes it from the Ca^{2+}-activated and auxin-regulated anion currents (Schroeder and Hagiwara, 1989; Marten et al., 1991). Alternatively, it could result from Ca^{2+} influx, which is known to sometimes occur in response to ABA (Schroeder and Hagiwara, 1990). Patch clamp experiments to determine the ionic species responsible for this "leak" current and its effect on Ca^{2+}_i would be helpful in further evaluation of the origin of Ca^{2+}_i.

Can elevated Ca^{2+}_i be bypassed in some responses to ABA? Results of imaging studies reveal that ABA-induced stomatal closure is not invariably associated with elevated Ca^{2+}_i in stomata of *C. communis* (Gilroy et al., 1991; McAinsh et al., 1992). Thus, either detection of an ABA-stimulated Ca^{2+}_i increase is limited by current technology (McAinsh et al., 1992), or ABA works through two pathways: Ca^{2+}-independent and Ca^{2+}-dependent or Ca^{2+}-accelerated (MacRobbie, 1990; Gilroy et al., 1991). One Ca^{2+}-independent pathway may involve cytosolic pH; elevated pH_i enhances outward K^+ currents from *V. faba* GCs (Blatt, 1992), an effect also caused by ABA (Blatt, 1990) and associated with stomatal closure.

EFFECTORS

Effectors produce a response at the end of a signal transduction chain. In GCs, these molecules are H^+-ATPases, K^+ channels, and anion channels, which direct the flow of ionic osmotica to swell or shrink the cells.

H^+-ATPases

Plasma membrane H^+-ATPases are effectors of stomatal opening. GC H^+-ATPases are activated by auxins (Lohse and Hedrich, 1992), blue light (Assmann et al., 1985; Schroeder, 1988), and red light (Serrano et al., 1988). It is not yet known whether the same H^+-ATPase isoform is the target of converging signal transduction pathways, or whether each signal activates a unique H^+-ATPase isoform.

K^+ Channels

The predominant osmotic effectors of stomatal opening and closing are inward and outward K^+ channels, respectively (Schroeder, 1988). "Inwardly rectifying," or "inward," K^+ channels are gated by voltage and K^+ concentrations so that they open under conditions where the electrochemical gradient drives K^+ influx through the channels. "Outward" K^+ channels are modulated by voltage and extracellular K^+ concentrations so as to mediate K^+ efflux. Regulation of K^+ currents by G proteins, IP_3, Ca^{2+}, and pH were discussed above.

Anion Channels

Three different types of anion channels have been identified in GCs. The R type (for rapid), regulated by auxins, rapidly activate and deactivate within their voltage range, and inactivate following prolonged stimulation (Marten et al., 1991; Schroeder and Keller, 1992). Because the voltage range for R-type channels is positive of −80 mV with a maximum current at −30 mV, in vivo activation of these channels may first require another mechanism to depolarize the membrane potential into their active range. This depolarization might be provided by the second type of anion channel, which is stretch-activated (Cosgrove and Hedrich, 1991), or by Ca^{2+} channels. The third type of anion channel, S type, has a maximum current at 0 mV, but the voltage range extends to voltages as negative as −140 mV. S-type channels activate and deactivate very slowly and do not inactivate (Schroeder and Hagiwara, 1989; Schroeder and Keller, 1992). Because they do not inactivate, S-type anion channels may be most important for stomatal closure, which requires lengthy anion efflux.

CONCLUSION

Despite a recent proliferation of studies on stomatal function, much remains unknown. The unique cell wall structure of GCs provides an interesting system for studying cell wall biosynthesis and regulation. Identification of signal receptors is of obvious importance, and the status of Ca^{2+} stores and interactions with ABA will be clarified by further research. Tantalizing "leak" currents in ABA and IP_3 responses need identification. The regulation and importance of photosynthetic carbon metabolism in GCs will benefit from closer scrutiny. Correlations between redox chains and ATPases, and between stomatal aperture and *E* should be given mechanistic underpinnings or put aside. Finally, research is needed to determine whether paradigms of stomatal dynamics in *V. faba* hold true in other species. These endeavors will eventually lead to an intricate and beautiful picture of stomatal response to the environment. For now, we have Figure 1.

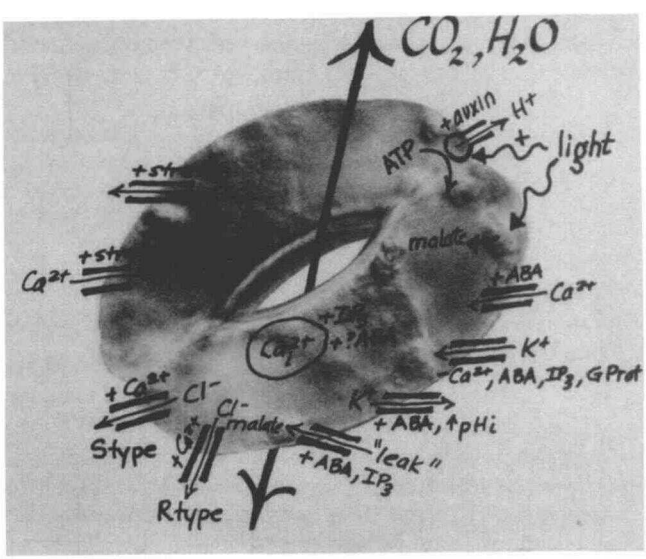

Figure 1. Effectors of osmotic potential in GCs and their regulators. Ions carried by the channels are indicated at the base of the flux arrows. Positive regulation is represented by a "+" and negative regulation by a "−". In some cases, regulation is indirect. See the text for discussion of the signal transduction chains underlying these effects.

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